

Composition and Distribution of *Pythium* Communities in Wheat Fields in Eastern Washington State

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ABSTRACT

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Pythium spp. were isolated from a mixture of soil and roots collected from 80 wheat fields in eastern Washington in the summer of 2000 from an area encompassing approximately 27,000 km². These sites covered a range of soil textures (coarse to fine, silty loess), average annual precipitation (200 to 600 mm), and average annual temperatures (7 to 11°C). Soil type and annual precipitation run in an east-west gradient, while temperature has a north-south gradient. Species were identified using classical methods and by sequencing the internal transcribed spacer (ITS)-1 region of the rDNA and comparing these sequences to a database from a worldwide collection of *Pythium* spp. The species with the highest frequency of occurrence among all the sites were *P. abappressorium* sp. nov. (A) (50%), *P. rostratum* (R) (40%), *P. debaryanum* (D) (37.5%), *P. heterothallicum* (H) (33.7%), *P. oligandrum* (O) (31.2%), an unidentified *P. sp.* (aff. *echinulatum*) (E) (25%), and *P. ultimum* (U) (18%). *P. intermedium*, *P. irregulare*, *P. paroecandrum*, *P. sylvaticum*, *P. dissimile*, and *P. dissotium* were isolated at a low frequency. From one to six species were isolated at each site, and there were 46 different species

combinations detected. The species presence/absence data from all sites were analyzed with Jaccard's similarity coefficient hierarchical cluster analysis. Six communities were identified (species within each community designation in order of frequency among the sites within the community)—AD, AOU, AR, DEH, HE, and RU. In general, *P. abappressorium* was evenly distributed over all zones. AOU was more prevalent in zones with lower precipitation and coarser soil, while DEH and HE were associated with zones with higher precipitation and finer-textured soils on the basis of comparison of frequency distributions with the expected distribution over all the sites. The RU community was more prevalent in higher temperature zones. Canonical correspondence analysis was performed to examine the relationship between species and environmental variables. Soil type and precipitation were highly correlated with each other and with axis 1, which separated *P. ultimum* and *P. abappressorium* (lower variable values) from *P. heterothallicum* (higher variable values). Axis 2 and 3 were most correlated with temperature, and these axes separated *P. oligandrum* (higher value) from *P. debaryanum* (lower value) and *P. ultimum*-*P. rostratum* from the other species. The results suggest that *Pythium* species composition, distributions, and associations on a given crop may be influenced by environmental factors at a mesoscale level (100 to 1,000,000 ha).

Pythium root rot is an important yield-limiting disease on wheat and barley produced under dryland conditions in eastern Washington (5,6). This disease is characterized by a reduction in seedling emergence, smaller and distorted first true leaves, plant stunting, reduced tillering, loss of fine feeder roots, and lower yields. Because of the ubiquitous nature of *Pythium* spp. in most soils, Cook and Veseth (7) have referred to this disease as the "common cold" of wheat. *Pythium* spp. have also been reported to be a problem on wheat and barley in Australia (35) and the United Kingdom (46).

Despite the prevalence of this genus on wheat, little is known about the composition and distribution of species over the diverse habitats characterized by variable edaphic and environmental conditions in eastern Washington. This area is characterized by deep wind-deposited (loess) soils, a range in rainfall from 220 to 650 mm per year, and rolling hills in the most eastern part. Twenty-nine species of *Pythium* have been reported on wheat worldwide (Fungus-Host Distributions, Systematic Botany and Mycology Laboratory, USDA Agricultural Research Service, Beltsville, MD) and 19 have been reported in North America (12). Chamswarng and Cook (4) isolated 10 species of *Pythium* and two unidentified *Pythium* isolates, but they only sampled from fields in eastern Whitman County, WA and adjacent western Latah County, ID.

Pythium spp. can be difficult to identify using morphological characteristics. Many species are asexual or heterothallic and do not form sexual structures in culture. Within the last 10 years, molecular techniques have been used for the identification, taxonomy, and phylogeny of *Pythium* spp., especially techniques based on the internal transcribed spacer (ITS) region of the ribosomal DNA (21,24,25,36,47). Paulitz et al. (37) described a new species of *Pythium* from wheat in eastern Washington based on morphological traits and unique sequence of the ITS region.

The influences of environmental factors on *Pythium* communities and species have not been examined in detail at the mesoscale level. Some studies (13,14) have examined soil factors associated with suppression of *Pythium*, such as soil C, N, and microbial activity. Other studies have examined correlations among selected environmental factors and frequency of *Pythium* species at a few field sites (23). In this study, we attempted to characterize the distribution of *Pythium* species over a range of soil and climatic zones over eastern Washington. Because of the topography and geography of this region, there is a gradient of soil type, annual precipitation, and annual temperature across the dryland wheat-growing area from central Washington to the Idaho border.

Multivariate statistics are widely used in ecology (20) and offer tools to examine the relationships among multiple environmental variables and species composition. For instance, these techniques have been used in soil ecology to identify factors that influence populations of soil microorganisms or to characterize populations of microbes in response to different soil management treatments, such as conventional and reduced tillage. These techniques include principal components analysis (34,40), discriminant analysis

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(11), and canonical discriminant analysis (14). One recent technique that has proven useful in plant and insect ecology but has not been applied in plant pathology studies is canonical correspondence analysis (CCA) (41). CCA is a type of direct gradient analysis, which examines the relationship between two sets of variables by constructing axes based on linear combinations of variables. Variables can then be ordinated on a two-dimensional graph. This technique is similar to principal components analysis and uses an eigenanalysis of a correlation matrix. This technique has been used to compare microbial populations (17) and soil fauna (10) in conventional and reduced-input farming systems and to compare composition of nematodes and fungal populations on the roots of a sand dune grass (43).

The purpose of this paper was to survey *Pythium* communities from wheat fields over a range of soil and climatic zones in eastern Washington. Communities were defined based on assemblages of species within and among sites. Species were identified using classical and molecular techniques, and multivariate techniques were used to examine species associations and correlations of communities with soil type, precipitation, and temperature across this region.

MATERIALS AND METHODS

Collection and maintenance of isolates. Soil and root samples were collected from 80 wheat fields representing over 27,000 km² in eastern Washington from July to August 2000 (Fig. 1). Soil from the top 30-cm depth was collected with a shovel within rows of wheat from three sites in each field for a total of approximately 2 kg. Samples were stored in plastic bags at 4°C until processing. Three 20-g samples containing roots and soils were removed from each bag, placed in 15-cm petri dishes, rewetted with 2 to 3 ml of water (approximate field capacity), and incubated at 22°C for 24 to 48 h. Plates were flooded with distilled water, and 10 grass clippings (approximately 1 cm in length) (*Poa pratensis* L.) were floated on the water. After 24 to 36 h incubation, the grass clippings were blotted on sterile paper towels and plated on a modified *Pythium*-selective medium (33) using water agar instead of corn meal agar. Emerging hyphal tips were removed from all colonies 1 day later using a pasteur pipette drawn out to a fine tip and plated on water agar. Cultures were also grown on potato dextrose agar (PDA) and V8 agar and later transferred to autoclaved grass clippings in sterile pond water for identifications (42). Cultures were stored at 4°C on PDA slants and sterile oat seeds. *Pythium* species were identified using the keys and descriptions of van der Plaats-Niterink (42), Dick (8), Matthews (30), Middleton (32), and Waterhouse (48).

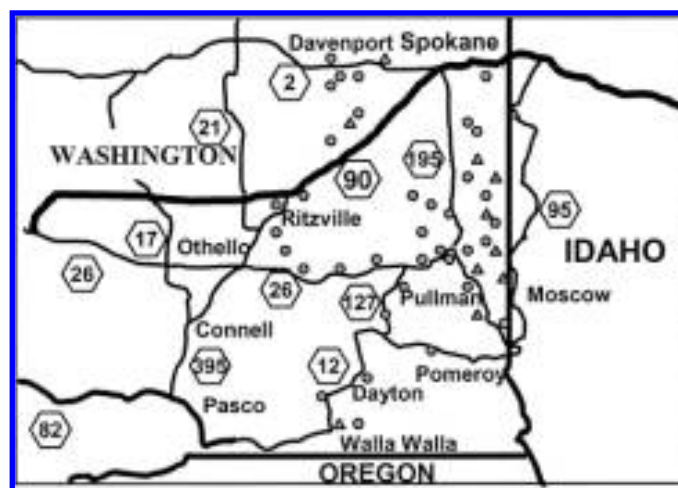


Fig. 1. Map of eastern Washington showing sampling sites. Triangles represent multiple sampling sites.

Extraction, polymerase chain reaction (PCR) amplification, and sequencing of DNA. A random sample of the collection, approximately 200 isolates, was selected for ITS identification. DNA was isolated, amplified, and sequenced according to the methods described in Paulitz et al. (37). Mycelium was grown in potato dextrose broth in 12.5 × 1.5-cm tubes at room temperature on an orbital shaker at ca. 110 rpm. DNA was extracted from mycelium using FastDNA tubes (FastDNA Kit, Bio101, Carlsbad, CA), CLS-Y cell lysis solution, and homogenization in an FP 120 FastPrep Cell Disruptor (Thermo Savant, Holbrook, NY) at speed 4 for 40 s. Samples were then centrifuged at 12,000 rpm for 1 min, the supernatant transferred to 1.5-ml microcentrifuge tubes and mixed with 600 µl of binding matrix. After incubation for 5 min at room temperature, the tubes were centrifuged at 12,000 rpm for 1 min, and the supernatant was discarded. Pellets were resuspended in 500-µl salt/ethanol wash solution (SEWS)-M and centrifuged at 13,000 rpm for 1 min. After removal of the wash solution, tubes were spun 5 s, and the residual solution was pipetted off before eluting DNA. The matrix was resuspended in 100-µl DNA elution solution (DES) and incubated for 3 min at room temperature. Samples were then centrifuged at 12,000 rpm for 1 min, and the supernatant was transferred to 0.5-ml microcentrifuge tubes.

DNA was amplified with ITS-1 primers UN-UP18542 and OOM-LO5.8S47B and ITS-2 primers OOM-UP5.8S01 and PY-LO28S22 (24) using the reaction mixtures and cycling conditions of Paulitz et al. (37). PCR products were treated with ExoSAP-IT (United States Biochemical, Cleveland, OH) and reamplified with Big Dye Mix (ABI-Prism, Foster City, CA). After cycling, the sequencing reaction was cleaned over a Sephadex G-50 fine mini spin column, dried in a speed-vac, resuspended in loading dye, and run on an ABI 377 sequencer. The ITS-1 DNA sequences were compared using BLAST with over 1,200 sequences from a worldwide collection that included types and neotypes of most species of *Pythium* (C. A. Lévesque, unpublished data).

Mapping of sample sites and characterization of environmental zones. Each sample site was mapped on a 1:150,000 topographical map and later on a U.S. Geological Survey (USGS) 1:24,000 topographical map using Maptech software (Maptech, Andover, MA). Each sample site was located on the appropriate maps and classified according to soil type, precipitation, and temperature zone. The soil type of each site was classified using the general soil map of Washington (3). Typically, the soils were deep, loessial, silty soils that have been wind-deposited over thousands of years. Soil texture varied along a generally east-west gradient, with the coarsest soils in the west (L2, dry, coarse-silty, loessial soil) and the finer soils in the east (L5, fine-silty, loessial soil). The soils had evolved under shrub-steppe vegetation in the west and under meadow-steppe vegetation in the east. L4 and L5 soils were also humus-rich. L2 soil is an aridic/mesic soil and L3, L4, and L5 are xeric/mesic soils. All soil series are classified as Mollisols. Average annual precipitation and average annual temperature zones were assigned from interpolated maps (28). These maps were derived using geostatistical methods of squared inverse distance weighing (IDW) to interpolate weather data (30-year averages) from weather stations to a regular grid of points 20 km apart. There were four annual precipitation zones (200 to 300, 300 to 400, 400 to 500, and 500 to 600 mm) and five average annual temperature zones (7 to 8, 8 to 9, 9 to 10, 10 to 11, and 11 to 12°C).

Statistical analyses. Because the baiting technique was not quantitative within a sample, only the presence or absence of each species was recorded for each site. The frequency of occurrence of each species was calculated as the number of sites that contained that species/total number of sites × 100. Species richness of each site was determined as the total number of *Pythium* species detected at each site. Relationships between species richness and environmental variables were evaluated by regression analysis (JMP Version 4.0, SAS Institute, Cary, NC).

To group species into communities, hierarchical cluster analysis was performed using Jaccard's similarity coefficient calculated for each possible pairwise combination of sampling sites (27), such that $C_j = j/(a + b + j)$, where j = the number of species in common to a pair of sampling sites A and B, a = the number of species unique to sample site A, and b = the number of species unique to sample site B. This index, also known as Jaccard's similarity index, is widely used in ecology with presence-absence data (26,45). Two sites will be more similar if they share more species and more dissimilar if more species are unique to one site. Dendrograms were constructed using the unweighted pair group method using arithmetic averages (UPGMA) method (38).

CCA was used to examine the relationship among species and environmental factors (41). Two matrices were used. The species matrix consisted of the species composition at each of the 80 sites, and the environmental matrix was composed of the three environmental variables as ordinal data at each site (soil class 2-5, precipitation class 2-5, and temperature class 7-11). A weighted correlation was calculated among the three environmental variables. The statistical significance of eigenvalues and species-environment correlations within each axis were tested with a Monte Carlo simulation of 1,000 runs. The resulting P value was computed as the proportion of randomized runs with an eigenvalue greater than or equal to the observed. Cluster analysis and CCA were performed using the PC-ORD software version 4. 20 (MJM Software Design Gleneden Beach, OR).

For each *Pythium* community identified from the cluster analysis, the median and mean values for each environmental factor were calculated based on all the sites classified in that community. To determine whether the frequency distributions of environmental classes varied among the *Pythium* communities, the following test was performed. An expected frequency distribution was constructed for each variable based on the total number of sampling sites (80). An observed frequency distribution for each environmental variable was derived for each community based on the sampling sites classified within that community. For example, 61.2% of the total sample sites were classified in soil L5, 11.2% in soil L4, 20% in soil L3, and 7.5% in soil L2. This would be the expected frequency distribution for the soil variable. The observed and expected frequency distributions were compared using a Kolmogorov-Smirnov test, which calculated a P value from a two-tailed Chi-Square approximation (Statistix Version 7, Analytical Software, Tallahassee, FL).

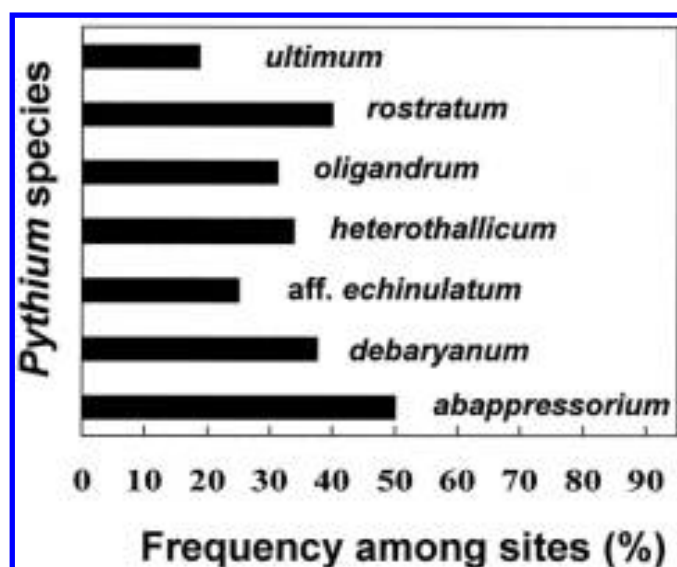


Fig. 2. Frequency of occurrence of *Pythium* species from 80 sites in eastern Washington.

RESULTS

Species composition. Fourteen species of *Pythium* were isolated from the 80 sample sites, from a total of 532 isolates. The most common species among sites were *Pythium abappressorium* sp. nov. Paulitz & Mazzola, *P. debaryanum* Auct. non Hesse, *P. heterothallicum* Campbell & Hendrix, *P. oligandrum* Drechs., *P. rostratum* E.J. Butler, and *P. ultimum* var. *ultimum* Trow. One unidentified species was found, which formed large liniform sporangia 20 to 32 μ m in diameter, often in doublets. The colonies of this species formed chrysanthemum patterns on PDA, with a colony growth rate of 5 to 6 mm per day at 25°C. Oospores were not formed in single culture, and attempts at mating were not successful. Based on ITS-1 sequences, the closest match was to *P. echinulatum*, and we therefore, assigned the name of *Pythium* sp. (aff. *echinulatum*). Seven other species were found at lower frequencies (number of sites in parentheses): *P. intermedium* de Bary (three), *P. irregulare* Buisman (four), *P. paroecandrum* Drechs. (four), *P. sylvaticum* Campbell & Hendrix (two), *P. dissimile* Vaartaja (one), *P. pleroticum* Ito (one), and *P. dissoticum* Drechs. (one). *P. dissimile* and *P. pleroticum* have not been reported from wheat before. *P. abappressorium* was the most common among sites, isolated from 50% of the sampled sites (Fig. 2). *P. debaryanum*, *P. rostratum*, and *P. heterothallicum* were found in 30 to 40% of the sites. *P. ultimum* was the least common of this group, being found in only 18% of the sites. The average species richness over all sites was 2.52 (SD 1.36), range 1-6, median = 2, and mode = 2. Species richness was not affected by soil type, precipitation, or temperature.

***Pythium* communities.** Four major communities were identified based on a dendrogram using Jaccard's similarity coefficient (Fig. 3). Within the largest community, all sites contained *P. abappressorium* (Cluster 1). This community could be divided into three subclusters, namely, *P. abappressorium* in association with *P. debaryanum* (Cluster 1A), *P. abappressorium* in association with *P. oligandrum* and *P. ultimum* (Cluster 1B), and *P. abappressorium* in association with *P. rostratum* (Cluster 1C). The other three main clusters contained *P. debaryanum* in association

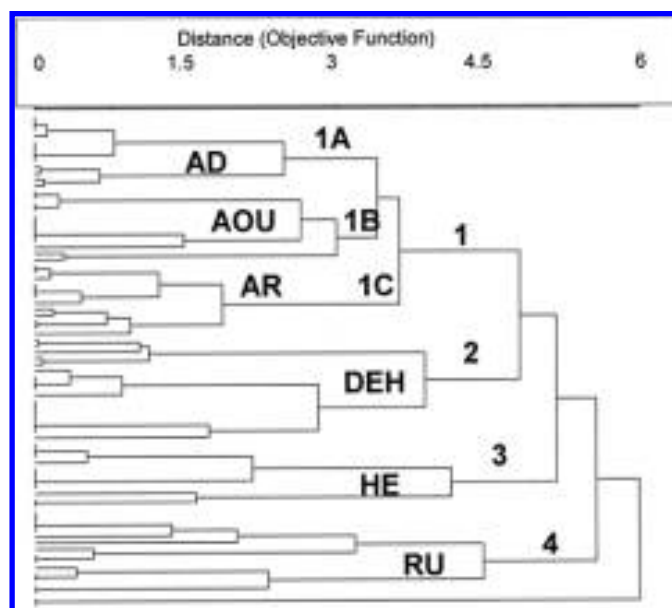


Fig. 3. Cluster analysis of sampling sites based on *Pythium* species present at each site and pairwise comparisons based on Jaccard's similarity coefficient. Dendrogram was constructed using the unweighted pair group method using arithmetic averages (UPGMA) method. Abbreviations indicate species composition of clusters. A = *P. abappressorium*; D = *P. debaryanum*; E = *Pythium* sp. (aff. *echinulatum*); H = *P. heterothallicum*; O = *P. oligandrum*; R = *P. rostratum*; and U = *P. ultimum*.

with *P. heterothallicum* and *P. sp. (aff. echinulatum)* (Cluster 2), *P. heterothallicum* in association with *P. sp. (aff. echinulatum)* (Cluster 3), and *P. rostratum* in association with *P. ultimum* (Cluster 4).

Relationships among environmental zones and *Pythium* communities. Soil type and annual precipitation were highly correlated ($r = 0.869$, $P = 0.000$), but average annual temperature and soil type ($r = 0.164$) and temperature and precipitation ($r = -0.071$) were not correlated. CCA identified three axes (Fig. 4 and 5). Axis 1 was highly correlated with soil and precipitation, and axis 2 and 3 were most correlated with temperature (Table 1). The eigenvalues of each axis were low (Table 2), explaining only 4.8, 1.9, and 1.2% of the variation for axis 1, 2, and 3, respectively. Eigenvalues represent the variance in the community matrix that is attributed to a particular axis. A Monte-Carlo randomization test of the eigenvalues showed that less than 3% of the 1,000 runs were greater than the observed values (Table 2). There was a significant correlation between sample scores for an axis derived from the species data and the sample scores that are linear combinations of the environmental variables (Table 3). For example, on axis 1, which was correlated with soil and precipitation, *P. ultimum* and *P. abappressorium* were separated in a direction opposite from that of *P. heterothallicum* (Fig. 4); the rest of the species clustered toward the origin (center of graph). On axis 2, which showed the strongest correlation with temperature, *P. debaryanum* was separated from *P. oligandrum*. On axis 3, which had the strongest correlation with temperature, *P. ultimum* and *P. rostratum* were separated in the opposite direction compared to the rest of the species (Fig. 5).

Four of the communities were not distributed as expected among the environmental classes. The *P. abappressorium*-*oligandrum*-*ultimum* community (Cluster 1B) was more predominant in lighter (coarser-textured) soils (L3) and drier precipitation zones (300 to 400 mm per year) (Table 4). The *P. debaryanum* and *P. heterothallicum* communities were more predominant than expected in the heavier (finer-textured) soils (L5) and zones with higher precipitation (500 to 600 mm). The *P. rostratum*-*ultimum*

community was strongly associated with the higher average annual temperature zone of 9-10°C.

DISCUSSION

This work is the first to show the species composition of *Pythium* communities in dryland wheat fields over a wide geographical area (27,000 km²) encompassing a range of soil, precipitation, and temperature zones. From the 80 sites, a total of 46 different species combinations were detected. This species variability may be due to sampling error or limits in detection, i.e., more species may be present but were not detected. From one to six species of *Pythium* were isolated from each site, which is less than the modal number of four to six species per site found by Dick and Ali-Shtayeh (9). Similarly, a small number of *Pythium* species predominated at each site in a survey of commercial alfalfa fields (15). In our survey, the most dominant species was the newly described *P. abappressorium*, which was isolated from half of the sites (37). This species forms small, plerotic oospores and spherical sporangia within appressoria with the remnants of the appressoria often remaining attached to reproductive structures in older cultures. This species was also isolated from apple roots in central Washington (31), being called *Pythium* MMI (aff. *Macrosporium*), but was shown to have ITS-1 sequences identical to *P. abappressorium* (C. A. Lévesque, personal communication). *Pythium* MMI was not pathogenic to apple, but precolonization of apple roots with this isolate provided biological control against pathogenic *P. ultimum* and *P. sylvaticum* (31).

It is unclear why *P. abappressorium* was not found in a previous survey of fields around Pullman, WA and Moscow, ID (4). *P. abappressorium* could have been mistaken for asexual forms of *P. ultimum* since both form similar-looking hyphal swellings. Chamswang and Cook (4) found isolates of *P. aristosporum* Drechs., *P. ultimum* var. *ultimum*, *P. ultimum* var. *sporangiferum*, *P. irregulare*, *P. torulosum* Coker & Patterson, *P. sylvaticum*, *P. volutum* Vanterpool, *P. heterothallicum* and two unidentified

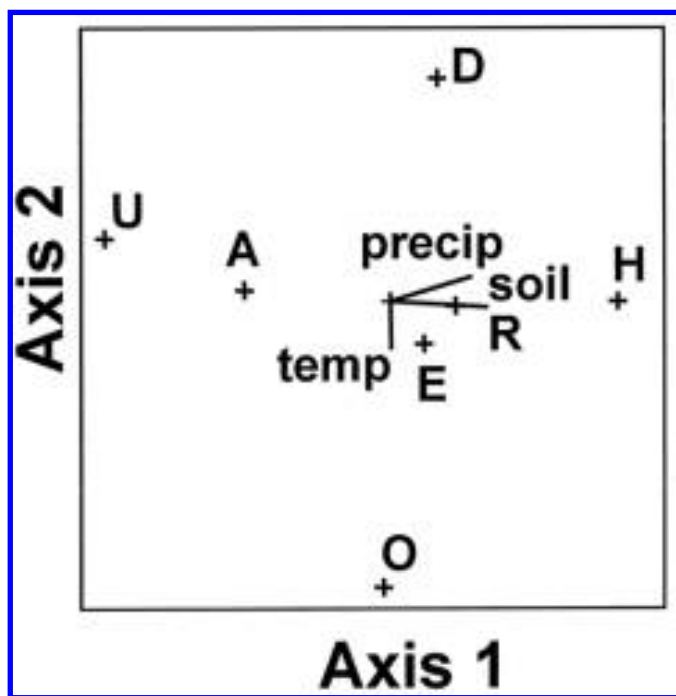


Fig. 4. Biplot from canonical correspondence analysis showing relationship between *Pythium* species and environmental factors (soil type, average annual precipitation, and average annual temperature) along Axis 1 and 2. Solid lines represent variable vectors. A = *P. abappressorium*; D = *P. debaryanum*; E = *Pythium* sp. (aff. *echinulatum*); H = *P. heterothallicum*; O = *P. oligandrum*; R = *P. rostratum*; and U = *P. ultimum*.

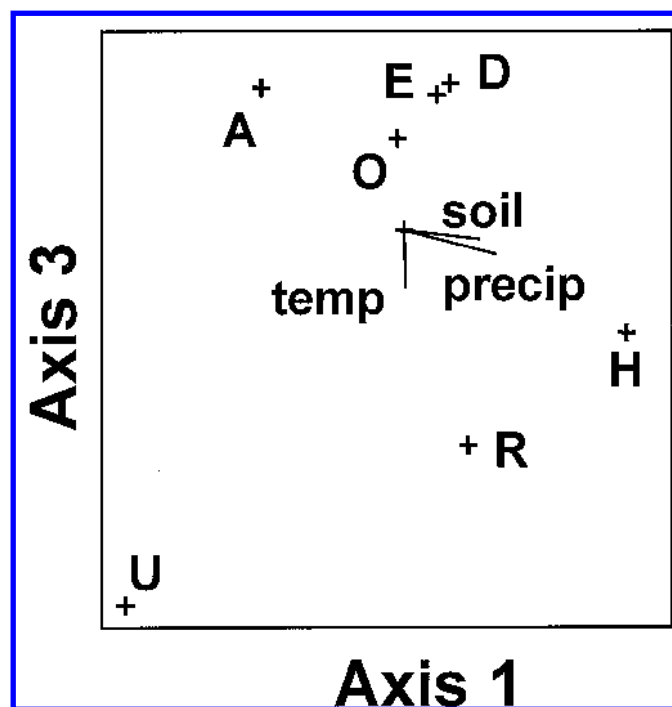


Fig. 5. Biplot from canonical correspondence analysis showing relationship between *Pythium* species and environmental factors (soil type, average annual precipitation, and average annual temperature) along Axis 1 and 3. Solid lines represent variable vectors. A = *P. abappressorium*; D = *P. debaryanum*; E = *Pythium* sp. (aff. *echinulatum*); H = *P. heterothallicum*; O = *P. oligandrum*; R = *P. rostratum*; and U = *P. ultimum*.

species, referred to as *Pythium* sp. “E” and “D”. *P. debaryanum* was common in our survey but absent from the previous survey of Chamswarng and Cook (4). It may have been classified as *P. irregulare* or *P. ultimum* in the previous survey since the key of van der Plaats-Niterink (42) did not recognize *P. debaryanum* as a valid species and these three species are morphologically similar. The low frequency of *P. sylvaticum* and *P. irregulare* in our survey is also in contrast with the results of surveys of Chamswarng and Cook (4) and the work of Pankhurst et al. (35), who found *P. irregulare* to be the dominant species in wheat field soils in south Australia. In a survey of *Pythium* spp. on perennial grasses, *P. irregulare-debaryanum*, *P. torulosum*, and heterothallic forms were the most predominant of 431 isolates (18). *P. rostratum* was common in our survey, but it was not detected by Chamswarng and Cook (4). Sprague (39) specifically mentioned *P. rostratum* as having been found in the Pullman, WA area. Our isolates of *P. rostratum* did not completely match the type species based on ITS-1 sequences, but it closely resembled this species based on its morphological description. Mazzola et al. (31) isolated an identical fungus based on ITS-1 sequences, which they termed *Pythium* MM4 (aff. *rostratum*).

P. ultimum was isolated only from roughly 20% of the sites. Chamswarng and Cook (4) found *P. ultimum* made up less than 5% of isolates from a single site. The sporangia of our *Pythium* sp. (aff. *echinulatum*) resemble those labeled by Chamswarng and Cook (4) as *Pythium* sp. “E” and described as heterothallic (did not form oospores in single culture). However, we were unable to induce oospore formation in *Pythium* sp. (aff. *echinulatum*), even with limited pairing of isolates.

Based on limited virulence testing of isolates from our survey, *P. ultimum*, *P. debaryanum*, and *P. irregulare* were the most virulent, causing significant damping-off of wheat, followed by *P. abappressorium* and *P. rostratum* (R. Higginbotham, T. Paulitz, and K. Kidwell, unpublished data). *P. heterothallicum* and *Pythium* sp. (aff. *echinulatum*) did not cause damping-off but did reduce root biomass of wheat. Ingram and Cook (19) found that *P. ultimum* and *P. irregulare* significantly reduced emergence and length of the first true leaf of wheat and that *P. heterothallicum* was only weakly pathogenic.

P. graminicola and *P. arrhenomanes* have been implicated in root rot of wheat in the United Kingdom (46) and in the North American Great Plains of the United States (44). *P. arrhenomanes* is also pathogenic on oats and barley (22) and is one of the most prevalent and aggressive pathogens of turfgrass (1). However,

TABLE 1. Intraset correlation and biplot scores between environmental factors and axes of canonical correspondence analysis

Variable	Correlations (biplot scores)		
	Axis 1	Axis 2	Axis 3
Soil	0.982 (0.295)	−0.113 (−0.022)	−0.150 (−0.023)
Precipitation	0.873 (0.262)	0.350 (0.067)	−0.339 (−0.051)
Temperature	−0.019 (−0.006)	−0.772 (−0.147)	−0.635 (−0.095)

TABLE 2. Eigenvalues from matrix used in canonical correspondence analysis

Axis	Real data	Randomized data Monte Carlo test			
	Eigenvalue	Mean	Minimum	Maximum	<i>P</i> ^a
1	0.090	0.040	0.011	0.101	0.007
2	0.036	0.018	0.001	0.051	0.030
3	0.023	0.006	0.000	0.027	0.005

^a *P* = proportion of randomized runs with eigenvalue greater than or equal to the observed eigenvalue, i.e., $P = 1 + \text{no. permutations} \geq \text{observed} / (1 + \text{no. permutations})$. Monte Carlo test based on 999 runs.

these pathogens have been notably absent in surveys of wheat in the dryland Pacific Northwest (PNW). One notable distinction is that the climate of the PNW is Mediterranean, with warm, dry summers, unlike the other areas that receive considerable precipitation during the summer growing season.

Two other considerations must be taken in interpreting this survey: the time of sampling and the method of isolation. We sampled toward the end of the growing season in July and August when soil conditions were fairly dry, unlike Chamswarng and Cook (4), who sampled in the fall and spring. We also baited with grass leaves, which may provide a wider diversity of *Pythium* spp. than methods that isolate directly from wheat roots or from soil dilution plates, especially those species that are weakly or non-pathogenic on wheat. It is difficult to isolate *Pythium* spp. from roots of mature wheat plants because diseased roots are rotted away and not recovered during root washing and due to competition from secondary colonists. Before baiting, we wetted and incubated the soils for a few days so our isolations probably reflect the species of *Pythium* that would be active at the start of the wet season, when winter wheat is planted in the fall. By collecting all the samples from dry soil and roots toward the end of the season, we also avoided the confounding factor of time of isolation from living roots. For example, some species may be more dominant from roots in the late fall-early spring, but less dominant later in the season. If samples were collected over a range of times, it would be difficult to compare among sites.

Few studies have examined the composition of *Pythium* communities in soil, especially over a large area. Dick and Ali-Shtayeh (9) examined communities of *Pythium* spp. in 38 farmland and parkland sites near Reading, England, but their study was done on a number of soil types over a small geographical area. They identified a number of communities with cluster analysis and found that land management (e.g., cultivation practices) had a greater influence than cropping sequence. In their study, *Pythium intermedium* and asexual *Pythium* spp. with hyphal swellings were the most abundant species. In our work, cluster analysis based on Jaccard's similarity coefficient defined six distinct com-

TABLE 3. Species-environment correlations from canonical correspondence analysis

Axis	Real data	Randomized data Monte Carlo test			
	Spp-Env. Corr.	Mean	Minimum	Maximum	<i>P</i> ^a
1	0.483	0.345	0.182	0.527	0.016
2	0.352	0.231	0.054	0.409	0.015
3	0.248	0.135	0.022	0.309	0.016

^a *P* = proportion of randomized runs with eigenvalue greater than or equal to the observed eigenvalue, i.e., $P = 1 + \text{no. permutations} \geq \text{observed} / (1 + \text{no. permutations})$. Monte Carlo test based on 999 runs.

TABLE 4. Environmental classification of *Pythium* communities according to soil type, precipitation, and temperature zones in eastern Washington

Community	Environmental variable		
	Soil type	Precipitation	Temperature
<i>P. abappressorium-debaryanum</i>	3.9 (4) ^a	3.7 (4)	8.1 (8)
<i>P. abappressorium-oligandrum-ultimum</i>	3.3 (3) ^{*b}	3.2 (3) [*]	8.0 (8)
<i>P. abappressorium-rostratum</i>	4.5 (5)	4.0 (4)	8.6 (8)
<i>P. debaryanum</i>	4.7 (5) [*]	4.5 (5) [*]	8.0 (8) [*]
<i>P. heterothallicum</i>	4.8 (5) [*]	4.4 (4) [*]	8.3 (8)
<i>P. rostratum-ultimum</i>	4.2 (5)	4.0 (4.5)	8.9 (9) [*]
Mean	4.2 (5)	4.0 (4)	8.3 (8)

^a Average and median (in parentheses) values for each environmental variable where each *Pythium* spp. occurred.

^b * denotes that the frequency distribution is significantly different from the expected ($P < 0.05$), based on Chi-Square approximation of two-tailed Kolmogorov-Smirnov statistic.

munities of *Pythium* spp. Three communities contained *P. abapressorium*, which was found in similar frequencies across all environmental zones. However, one subcommunity, *P. abapressorium* in association with *P. oligandrum* and *P. ultimum* did not fit the expected distribution, but was more predominant in coarser-textured soil and drier precipitation zones. Hancock (15) found a similar correlation with *Pythium ultimum* on alfalfa roots and soil temperature, with *P. ultimum* being more predominant in the warmer summer months. In our work, a Chi-square test showed a significant association between *P. oligandrum* and *P. ultimum*. This may be due to a mycoparasitic relationship (2) between the two fungi, or because they are adapted to similar environments. Two communities dominated by *P. debaryanum* and *P. heterothallicum*, respectively, were more prevalent than expected in the wetter precipitation zones and finer-textured soils. *P. debaryanum* and *P. sp.* (aff. *echinulatum*) were not detected in soil zone L2 and *Pythium sp.* (aff. *echinulatum*) and *P. heterothallicum* were not found in zones with 200 to 300 mm of precipitation per year. *P. heterothallicum* does not form oospores in single culture and may reproduce asexually in the field without a resistant oospore to survive drier conditions. *P. debaryanum* produces oospores readily. On the other hand, *P. ultimum* was found at a higher frequency in courser-textured, drier soils in the western part of the sampling zone, compared to the wetter finer-textured soils in the eastern part. These same trends were evident graphically in the biplot of the CCA. *P. ultimum* and *P. abapressorium* were separated in opposite directions from *P. debaryanum* and *P. heterothallicum* on axis 1, which was highly correlated with both soil type and precipitation. Both soil type and precipitation were also highly correlated with each other due to the prevailing weather patterns in the region, increasing clay content with increasing annual precipitation. The precipitation gradient is due to the rain shadow of the Cascade Mountains, with more precipitation to the east. The prevailing wind direction also runs west to east, so over thousands of years, larger soil particles were deposited in the western part of the sampling area with smaller soil particles in the eastern part. However, one confounding factor should be considered. In the lower rainfall zones, farmers practice a winter wheat-summer fallow rotation, where the field is not cropped every other year to accumulate soil moisture for the winter wheat. In addition, the fallow is often maintained with extensive cultivation using a rod weeder, which pulverizes the soil into a fine powder to break the capillary connection with the lower soil and inhibit soil water loss by evaporation. Farmers in the wetter areas practice annual cropping, often with a winter wheat wheat-spring barley-spring pea rotation. These differences in management practices may also influence the makeup of *Pythium* communities.

Other studies have similarly shown a correlation between environmental factors and *Pythium* spp. On alfalfa, *P. ultimum* and *P. irregulare* were often together and positively correlated with average rainfall (23). Conversely, *P. sylvaticum* was negatively correlated with rainfall and seemed to be associated with higher temperatures and drier conditions (23). However, this was based on only two field sites over 2 years. Soil texture differences have been associated with differences in *Pythium* activity. In the San Joaquin Valley of California, finer-textured silty clay and silty clay loams tended to be more suppressive to *P. ultimum* than coarser clay loam soils (29). However, soil texture alone was not responsible for this difference, which was attributed to the activity of *P. oligandrum*, which was more tolerant of high Cl levels and could compete saprophytically with *P. ultimum*. Hancock and Grimes (16) found a seasonal variation with *Pythium* on alfalfa in California, with *P. ultimum* being more prevalent in the summer and *P. irregulare* in cooler spring.

In conclusion, a number of *Pythium* communities and species associations were detected in wheat-field soils in eastern Washington and these were associated with environmental gradients across this region. *P. abapressorium* was detected at the most

sites and was widely distributed across all soil, precipitation, and temperature zones, while other communities dominated by species such as *P. debaryanum*, *P. heterothallicum*, and *P. sp.* (aff. *echinulatum*) were more frequent in wetter zones with finer-textured soils. Despite the detectable influence of environmental gradients on species composition, these three variables explained less than 10% of the variance in the sampled communities. Further work is needed to examine the temporal dynamics of *Pythium* communities, to see if these associations are present at other times of the year. Crop rotation and tillage practices may also drive the succession of these communities over time.

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